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Degradation of pharmaceuticals in non-sterile urban wastewater by *Trametes versicolor* in a fluidized bed bioreactor

Carles Cruz-Morató [a,1], Laura Ferrando-Climent [b,1], Sara Rodriguez-Mozaz [b], Damià Barceló [b,c], Ernest Marco-Urrea [a], Teresa Vicent [a], Montserrat Sarrà [a,*]

[a] Departament d'Enginyeria Química, Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Spain
[b] Catalan Institute for Water Research (ICRA), H_2O Building, Scientific and Technological Park of the University of Girona, 101-E-17003 Girona, Spain
[c] Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA), Spanish Council for Scientific Research (CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain

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ABSTRACT

The constant detection of pharmaceuticals (PhACs) in the environment demonstrates the inefficiency of conventional wastewater treatment plants to completely remove them from wastewaters. So far, many studies have shown the feasibility of using white rot fungi to remove these contaminants. However, none of them have studied the degradation of several PhACs in real urban wastewater under non-sterile conditions, where mixtures of contaminants presents at low concentrations (ng L^{-1} to μg L^{-1}) as well as other active microorganisms are present. In this work, a batch fluidized bed bioreactor was used to study, for the first time, the degradation of PhACs present in urban wastewaters at their pre-existent concentrations under non-sterile conditions. Glucose and ammonium tartrate were continuously supplied as carbon and nitrogen source, respectively, and pH was maintained at 4.5. Complete removal of 7 out of the 10 initially detected PhACs was achieved in non-sterile treatment, while only 2 were partially removed and 1 of the PhACs analyzed increased its concentration. In addition, Microtox test showed an important reduction of toxicity in the wastewater after the treatment.

1. Introduction

Pharmaceutical active compounds (PhACs) are emerging contaminants that have received much attention from the scientific community during the last 10 years due to their wide presence in the environment (Caliman and Gavrilescu, 2009; Mompelat et al., 2009). It is well known that their main route of entrance into the environment is via ingestion, following excretion and direct disposal via wastewater treatment plants (WWTP) and manufacturing (Daughton and Ternes, 1999). Conventional activated sludge technologies applied in WWTPs are not designed to remove these micropollutants, which are present at low concentrations (between ng L^{-1} and μg L^{-1}), and therefore they can pass through unchanged or

* Corresponding author.
E-mail address: Montserrat.Sarra@uab.cat (M. Sarrà).
[1] Carles Cruz-Morató and Laura Ferrando-Climent contributed equally to this work.
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partially transformed to the receiving environmental compartments (Verlicchi et al., 2012; Ratola et al., 2012). The possible negative ecotoxicological effect provoked by the presence of PhACs in the environment is an issue of environmental concern. Consequently, several investigators have focused on the potential risk of the presence of PhACs in different water compartments, which were recently reviewed by Santos et al. (2010), de Jongh et al., 2012 and Stuart et al., 2012. Although, chronic ecotoxicity data are scarce compared to acute studies, accumulative effects have been shown to damage some ecosystems (Daughton and Ternes, 1999).

Physico-chemical technologies such as advanced oxidation and photodegradation have been proposed as alternative approaches to achieve complete removal of some recalcitrant PhACs as carbamazepine and clofibric acid (Doll and Frimmel, 2004; Sirés et al., 2007; Esplugas et al., 2007). However, their main limitation is the formation of undesirable and sometimes toxic transformation products (Negrón-Encarnación and Arce, 2007). Alternatively, white rot fungi (WRF) have shown to be attractive candidates for designing effective bioremediation strategies of PhACs due to their unspecific oxidative enzymatic system, that includes lignin-modifiers enzymes, especially laccase and peroxidases, but also intracellular enzymatic complexes (e.g., cytochrome P450) (Asgher et al., 2008). Regarding the PhACs removal by WRF, fast degradation, from minutes to few days, has been demonstrated for β-blockers (Marco-Urrea et al., 2010a), some anti-inflammatory drugs (Marco-Urrea et al., 2010b, 2010c, 2010d), antibiotics (Rodríguez-Rodríguez et al., 2012; Prieto et al., 2011) and psychiatric drugs (Jelić et al., 2012), while other antimicrobial agents, estrogens (Cajthalm et al., 2009) and iodinated contrast agents (Rode and Müller, 1998) were removed at slower rates (more than a week). The main reactions involved in the transformation of pharmaceuticals by WRF include hydroxylation, formylation, deamination and dehalogenation (Cruz-Morato et al., 2013; Harms et al., 2011). Mineralization has been barely demonstrated, only suggested for some anti-inflammatory drugs (diclofenac and ketoprofen) (Marco-Urrea et al., 2010b and c). Ecotoxicological assessment of the treated effluents has to be performed, since the transformation products formed from the target contaminants during treatment may exhibit higher toxicity than the parent compound.

To date, most of the published studies on removal of PhACs by WRF were carried out in sterilized synthetic liquid media under controlled conditions of pH and temperature, with absence of competitors or spiking PhACs at concentrations higher than those found in real wastewaters (mg L^{-1}). As far as we know, the only work attempting the elimination of PhACs in non-sterile conditions was reported by Zhang and Geiβen (2012). They observed 60–80% of removal in the elimination of carbamazepine (spiked at 5 mg L^{-1}) in a bioreactor containing the WRF *Phanerochaete chrysosporium* immobilized in polyether foam and achieving a stable continuous operation during 100 days. However, degradation by WRF of PhAC mixtures at real concentrations in non-sterile wastewaters containing is still unproved.

The aim of this study is the use of a fluidized bed bioreactor inoculated with the WRF *Trametes versicolor* to degrade PhACs contained in urban wastewater at both sterilized and non-sterilized conditions. Previously to the batch reactor treatment at non-sterilized conditions, the requirements of nutrients in the real wastewater were studied.

2. Materials and methods

2.1. Fungus and chemicals

T. versicolor (ATCC#42530) was from the American Type Culture Collection and was maintained by subculturing on 2% malt extract agar slants (pH 4.5) at 25 °C. Subcultures were routinely made every 30 days.

Pellet production was done as previously described by Font et al. (2003). Pellets obtained by this process were washed with sterile deionized water.

All pharmaceutical standards were of high purity grade (>90%) and they were purchased from Sigma–Aldrich (Barcelona, Spain), European Pharmacopeia (EP) and Toronto research chemicals (Ontario, Canada).

The cartridges used for solid phase extraction were Oasis HLB (60 mg, 3 mL) from Waters Corporation (Milford, MA, USA). Glass fiber filters (1 μm) and nylon membrane filters (0.45 μm) were purchased from Whatman (U.K.). HPLC grade methanol, acetonitrile, water (Lichrosolv) and formic acid 98% were supplied by Merck (Darmstadt, Germany). Ammonium hydroxide and Ethylenediaminetetraacetic acid disodium salt solution (Na2EDTA) at 0.1 mol L^{-1} were from Panreac (Barcelona). Glucose, ammonium tartrate dibasic and malt extract were purchased from Sigma–Aldrich (Barcelona, Spain).

2.2. Urban wastewater samples

Urban wastewater samples were collected from the student's village of Universitat Autónoma de Barcelona (Spain). Table 1 shows the characteristics of the wastewater. Sample 1 was sterilized at 121 °C during 30 min. Samples 2 and 3 were directly used at non-sterile conditions.

2.3. Experimental procedures

2.3.1. Batch bioreactor treatment

A glass fluidized bed bioreactor with a useful volume of 10 L (Blánquez et al., 2008) was used to carry out both sterile and non-sterile urban wastewater treatments (wastewater samples 1 and 3, respectively). Approximately, 2.5 g dry weight

Table 1 – Characteristics of the urban wastewater samples from the university village.

Environmental parameter	Sample 1	Sample 2	Sample3
COD (mg L^{-1})	480	420	398
TOC (mg L^{-1})	105.8	135.3	116.02
$N-NH_4^+$ (mg L^{-1})	14.1	33.3	42
TS (mg L^{-1})	194	201	220
VS (mg L^{-1})	176	181	190.4
Conductivity (μg L^{-1})	287	731	552
pH	8.52	8.2	8.64